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ATTY.'S DOCKET: RUBINSTEIN=7

In re Application of:	Conf. No.: 2828
Menachem RUBINSTEIN et al	Art Unit: 1646
Appln. No.: 10/070,295	Examiner: G. Chandra
Date Filed: March 5, 2002	Washington, D.C.
For: USE OF LEPTIN IN INHIBITION OF ENDOTHELIAL CELL PROLIFERATION	

DECLARATION UNDER 37 CFR §1.132

Honorable Commissioner for Patents
U.S. Patent and Trademark Office
Customer Service Window
Randolph Building, Mail Stop
401 Dulany Street
Alexandria, VA 22314

Sir:

I, Menachem RUBINSTEIN, declare and state as follows:

I am a co-inventor in the above-identified application and my educational and professional experience is presented in the curriculum vitae attached hereto.

I understand that the examiner has made an enablement rejection and has stated that, while the specification teaches leptin inhibiting angiogenesis in ob/ob mice, there is no guidance or any other example that leptin would inhibit angiogenesis in a normal mammal. Thus, the examiner takes the position that one of skill in the art is

not enabled for inhibiting angiogenesis in mammals that are normal and not ob/ob.

The examiner's logic in the lack of enablement rejection however is faulty. One of the most convincing ways to prove the function of any given protein is to generate a knockout mouse, which is unable to express this protein. It is then customary to administer this protein in order to show that its biological activity is restored. There is a vast body of scientific literature, which is based on this paradigm and it is an accepted method by virtually all life scientists. Although fa/fa rats and ob/ob mice are phenotypically analogous, it is well established that ob/ob mice can be cured by exogenous administration of leptin, whereas fa/fa rats do not respond to leptin whatsoever. Therefore, the two rodent models differ phenotypically in the most relevant issue in the present application, i.e., response to leptin.

The ob/ob strain is a spontaneously generated leptin knockout mouse. It has a single point mutation in the leptin gene, which disrupts its open reading frame, resulting in leptin deficiency. Otherwise, this strain is genetically perfectly normal. The experimental results presented in the instant application demonstrate that the addition of leptin to a leptin-deficient (ob/ob) mouse resulted in induction of angiopoietin 2 and inhibition of

angiogenesis in its adipose tissue. As discussed above, this proves the antiangiogenic function of leptin in adipose tissue (It should be noted that angiopoietin 2 acts differently in the presence or absence of VEGF, where in the absence of VEGF, it will induce vascular regression and endothelial cell death, as shown in Fig. 2 of Hanahan, *Science* 277(5322):48-50 (1997), a copy of which is attached). However, in the case of a normal mouse (not leptin-deficient), the administration of leptin to a normal mouse is like bringing coal to Newcastle. It would make a little or no difference since normal adipose tissue makes its own leptin.

The Cao et al. reference cited by the examiner demonstrates that leptin induces capillary growth in the cornea model, although less than VEGF (Fig. 1). In the adipose tissue, absence of leptin in ob/ob mice reduced the extent of fenestration as compared with wild type mice (Fig. 3). The extent of fenestration induced by VEGF in the cornea is greater than that induced by leptin (Fig 2), yet leptin induced more leakage (Fig.4). Fenestration and capillary growth are two distinct processes. Cao et al. however did not measure capillary growth in the adipose tissue, but rather explained their observation of fewer capillaries in the adipose tissue of ob/ob mice as an outcome of larger adipocyte size and not due to lack of leptin (see page 6932, second

column, first paragraph). Thus, Cao certainly does not teach or suggest that leptin induces capillary growth in adipose tissue, as asserted by the examiner.

In conclusion, one of skill in the art would indeed be enabled for inhibiting angiogenesis in mammals as presently claimed.

The undersigned declares further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Jan 22nd, 2008
Date

Menachem Rubinstein
Menachem RUBINSTEIN

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1971CURRICULUM VITAE

Name : **RUBINSTEIN, Menachem**

Date & Place of Birth: 1945; Tel-Aviv, Israel.
Present home address: 1 Hohit St. Rehovot, Israel.

EDUCATION:

1966 - 1968	B.Sc. in Chemistry and Biochemistry, The Hebrew University, Jerusalem, Israel.
1969 - 1970	M.Sc. in Biochemistry, The Hebrew University, Jerusalem, Israel.
1971 - 1975	Ph.D., Department of Organic Chemistry The Weizmann Institute of Science, Rehovot, Israel. Mentor: Prof. Abraham Patchornik.

POSITIONS:

1976-1977	Postdoctoral Fellow, Dept. of Physiological Chemistry, Roche Institute of Molecular Biology. Nutley, New Jersey, USA. Mentor: The late Dr. Sidney Udenfriend
1978-1979	Visiting Scientist - Dept. of Physiological Chemistry and Pharmacology, Roche Institute of Molecular Biology.
1980-1983	Senior Scientist, Dept. of Virology, The Weizmann Institute of Science, Rehovot, Israel.
1983-1998 Genetics,	Associate Professor (with tenure) Dept. of Molecular The Weizmann Institute of Science.
1987-1990	Chief Scientist (R&D manager), InterPharm Laboratories, Kiryat-Weizmann, Nes-Ziona, Israel.
1990-1991	Visiting Associate Professor, Dept. of Pharmacology New-York University, Medical Center, New York, U.S.A.
1991-2000	Head, Biological Services, The Weizmann Institute of Science.

1998- present	Professor, Dept. of Molecular Genetics, The Weizmann Institute of Science.
2002-2004	Head, The postdoctoral Fellowship program, The Feinberg Graduate School The Weizmann Institute of Science.
2006 2008	Chair The Scientific Council The Weizmann Institute of Science.

HONORS:

1975	Landau Prize for Ph.D. Thesis.
1980	Ionel Saniel Career Development Chair.
1983	The Maurice and Edna Weiss Chair of Cytokine Research.
1996-1998	Member of the Editorial Board, Cytokines and Molec. Therapy.
1998-	Member of the Editorial Board, J. Interferon and Cytokine Res.
2000-	Member of the Editorial Board, Cytokine
2005	Recipient of the Milstein Award for IFN and Cytokine Research

PROFESSIONAL MILESTONES:

Development of HPLC methods for protein isolation and analysis	1977-1979
Isolation of opioid peptides and their precursors	1977-1978
Isolation and characterization of human interferon alpha	1978-1979
Characterization of the human interferon-γ receptor	1982-1986
Identification of soluble IFN-γ and IL-6 receptors in body fluids	1988-1990
Identification of a soluble LDL receptor as an antiviral protein	1989-1993
Characterization and molecular cloning of the Type I IFN receptor	1992-1994
Study of early events in signaling of the Type I IFN receptor	1995-1997
Leptin receptor signaling and leptin as an angiogenic modulator	1996-2005
Isolation and cloning of interleukin-18 binding protein	1997-2003
Regulation of IFN-gamma-induced gene expression	2000-present

LIST OF PUBLICATIONS

1. Rubinstein, M., DeGroot, N. and Lapidot, N. Peptidyl t-RNA. The chemical synthesis of peptidyl t-RNA containing hydroxy amino acids. Biochim. Biophys. Acta **209**, 183, 1970.

2. Rubinstein, M. and Patchornik, A. Polymers as chemical reagents. The use of poly(3,5-diethylstyrene) sulfonyl chloride for the synthesis of internucleotide bonds. Tetr. Letters 2881, 1972.

3. Hassner, A., Strand, G., Rubinstein, M. and Patchornik. A. Levulinic esters. An alcohol protecting group applicable to some nucleosides. J. Amer. Chem. Soc., **97**, 1614, 1975.

4. Rubinstein, M., Amit, B. and Patchornik, A. The use of a light-sensitive phosphate protecting group for some mononucleotide syntheses. Tetr. Letters 1445, 1975.

5. Rubinstein, M. and Patchornik, A. Poly(3,5-diethylstyrene) sulfonyl chloride. A new reagent for internucleotide bond synthesis. Tetrahedron **31**, 1517, 1975.

6. Rubinstein, M. and Patchornik, A. A novel method for phosphodiester and internucleotide bond synthesis. Tetrahedron **31**, 2107, 1975.

7. Shechter, Y., Rubinstein, M., Becker, R. and Bohak, Z. Modulating of the enzymic activity of chicken pepsin by the covalent modification of its single SH group. Europ. J. Biochem. **58**, 123, 1975.

8. Rubinstein, M., Shechter, Y. and Patchornik, A. Covalent chromatography the isolation of tryptophanyl containing peptides by novel polymeric reagents. Biochem. Biophys. Res. Comm. **70**, 1257, 1976.

9. Shechter, Y., Rubinstein, M. and Patchornik, A. Selective covalent binding of methionyl containing peptides and proteins to water insoluble polymeric reagents and their regeneration. Biochemistry **16**, 1424, 1977.

10. Rubinstein, M., Stein, S., Gerber, L.D. and Udenfriend, S. Isolation and characterization of opioid peptides from rat pituitary: β-Lipotropin. Proc. Natl. Acad. Sci. USA, **74**, 3052, 1977.

11. Rubinstein, M., Stein, S. and Udenfriend, S. Isolation and characterization of opioid peptides from rat pituitary: b-Endorphin. Proc. Natl. Acad. Sci. USA **74**, 4969, 1977.

12. Gerber, L.D., Stein, S., Rubinstein, M., Wideman, J. and Udenfriend, S. Binding assay for opioid peptides with neuroblastoma X glioma hybrid cells: Specificity of the Receptor Site. Brain Res. **151**, 117, 1978.

13. Rubinstein, M., Stein, S. and Udenfriend, S. Characterization of proopiocortin, a precursor to opioid peptides and corticotropin. Proc. Natl. Acad. Sci. USA **75**, 669, 1978.

14. Stein, S., Rubinstein, M., and Udenfriend, S. Ultramicro analysis of peptides. Psychopharmacology Bulletin (NIMH) **14**, 29, 1978.

15. Lewis, R.V., Stein, S., Gerber, L.D., Rubinstein, M. and Udenfriend, S. High molecular weight opioid containing proteins in the striatum. Proc. Natl. Acad. Sci. USA **75**, 4021, 1978.

16. Rubinstein, M., Rubinstein, S., Familletti, P.C., Gross, M.S., Miller, R.S., Waldman, A.A. and Pestka, S. Human leukocyte interferon purified to homogeneity. Science **202**, 1289-1290, 1978.

17. Rubinstein, M., Rubinstein, S., Familletti, P.C., Miller, R.S., Waldman, A.A. and Pestka, S. Human leukocyte interferon: Production, purification to homogeneity and initial characterization. Proc. Natl. Acad. Sci. USA **76**, 640-644, 1979.

18. Kimura, S., Lewis, R.V., Gerber, L.D., Brink. L., Rubinstein, M., Stein, S. and Udenfriend, S. Purification to homogeneity of camel pituitary pro-opiocortin: the common precursor of opioid peptides and corticotropin. Proc. Natl. Acad. Sci. USA **76**, 1756-1759, 1979.

19. Rubinstein, M., Stein, S., Chen-Kiang, S. and Udenfriend, S. Characterization of proteins and peptides by high performance liquid chromatography and fluorescence monitoring of their tryptic digests. Anal. Biochem. **95**, 117-121, 1979.

20. Rubinstein, M. Preparative high performance liquid chromatography of proteins. Anal. Biochem. **97**, 1-7, 1979.

21. Ortaldo, J.R., Pestka, S., Slease, R.B., Rubinstein, M. and Heberman, R.B. Augmentation of Human K-cell activity with interferon. Scan. J. Immunol. **12**, 365-369, 1980.

22. Levy, W.P., Shively, J., Rubinstein, M., del Valle, U. and Pestka, S. Amino-terminal amino acid sequence of human leukocyte interferon. Proc. Natl. Acad. Sci. USA **77**, 5102-5104, 1980.

23. Staehelin, T., Durrer, B., Schmidt, J. Takacs, B., Stocker, J., Miggiano, V., Stahli, C., Rubinstein, M., Levy, W.P., Hershberg, R. and Pestka, S. Production of hybridomas secreting monoclonal antibodies to the human leukocyte interferons. Proc. Natl. Acad. Sci. USA **78**, 1848-1852, 1981.

24. Herberman, R.B., Ortaldo, J.R., Rubinstein, M., Pestka, S. Augmentation of natural and antibody dependent cell mediated cytotoxicity by pure leukocyte interferon. J. Clin. Immunol. **1**, 149-153, 1981.

25. Rubinstein, M., Levy, W.P., Moschera, J.A., Lai, C.Y., Hershberg, R.D., Bartlett, R. and Pestka, S. Human leukocyte interferon: Isolation and characterization of several molecular forms. Archives of Biochemistry and Biophysics **210**, 307-318, 1981.

26. Evinger, M., Rubinstein, M. and Pestka, S. Antiproliferative and antiviral activities of human leukocyte interferons. Archives of Biochemistry and Biophysics **210**, 319-329, 1981.

27. Levy, W.P., Rubinstein, M., Shively, J., Del valle, U., Lai, C.Y., Moschera, J., Brink, L., Gerber, L., Stein, S. and Pestka, S. Amino acid sequence of a human leukocyte interferon. Proc. Natl. Acad. Sci. USA **78**, 6186-6190, 1981.

27b. Shiveli, JE, Del Valle, U., Blacher, R., Hawke, D., Levy, W.P., Rubinstein, M. Stein, S., McGregor, C., Tarnowsky, J., Bartlett, R., Lee, D., and Pestka, S., Microsequence analysis of peptides and proteins IV. Structural studies on human leukocyte interferon. Anal. Biochem. **126**, 318-326, 1981.

28. Orchansky, P., Rubinstein, M. and Sela, I. Human interferons protect plants from virus infection. Proc. Natl. Acad. Sci. USA **79**, 2278-2280, 1982.

29. Novick, D., Eshhar, Z. and Rubinstein, M. Monoclonal antibodies to human α-interferon and their use for affinity chromatography. J. Immunol. **129**, 2244-2247, 1982.

30. Fellous, M., Nir, U., Merlin, G., Rubinstein M., Wallach, D. and Revel. M. Interferon-dependent induction of mRNA for the major histocompatibility antigens in human fibroblasts and lymphoblastoid cells. Proc. Natl. Acad. Sci. USA **79**, 3082-3086, 1982.

31. Novick, D., Eshhar, Z., Gigi, O., Marks, Z., Revel, M. and Rubinstein, M. Affinity chromatography of human fibroblast interferon (IFN-β1) by monoclonal antibody columns. J. Gen. Virol. **64**, 905-910, 1983.

32. Ortaldo, J.R., Mantovani, A., Hobbs, D., Rubinstein, M., Pestka, S. and Herberman, R.B. Effects of Several Species of Human Leukocyte Interferon on Cytotoxic Activity of NK Cells and Monocytes. Int. J. Cancer **31**, 285-289, 1983.

33. Novick, D., Eshhar, Z., Fischer, D.G., Friedlander, J. and Rubinstein, M. Monoclonal antibodies to human interferon-γ: production, affinity purification and radioimmunoassay. The EMBO Journal **2**, 1527-1530, 1983.

34. Goren, T., Kapitkovsky, A., Kimchi A. and Rubinstein, M. High and low potency interferon-α induce (2'-5') oligo adenylate synthetase with similar efficiency. Virology **130**, 273-280, 1983.

35. Fischer, D.G., and Rubinstein, M. Spontaneous production of interferon-γ and acid labile interferon-α by a sub-population of human peripheral blood lymphocyte. Cellular Immunol. 81, 426-434, 1983.

36. Haimovitz, A., Fuks, Z., Rubinstein, M. and Treves, A.J. The instability of membrane markers expressed by human monocytes and macrophages in culture. Immunobiology **165**, 432-444, 1983.

37. Finel, M., Rubinstein, M. and Pick, U. Preparation of an ε-deficient chlorplast coupling factor 1 having a high ATPase activity. FEBS Letters **166**, 85-89, 1984.

38. Friedlander, J., Fischer, D.G. and Rubinstein, M. Isolation of two discrete human interferon-γ subtypes by high performance liquid chromatography. Analytical Biochemistry **137**, 115-119, 1984.

39. Orchansky, P., Novick, D., Fischer, D.G. and Rubinstein, M. Type I and Type II interferon receptors. J. of Interferon Research **4**, 275-282, 1984.

40. Chernajovsky, Y., Mory, Y., Chen, L., Marks, Z., Novick D., Rubinstein, M. and Revel, M. Efficient constitutive production of human fibroblast interferon by hamster cells transformed with the IFN-β_1 gene fused to an early SV40 promoter. DNA **3**, 297-308, 1984.

41. Weinberger, A., Fischer, D.G., Rubinstein, M., Weesenbeck, A., and Pinkas, J. A crossover study comparing diflunisal to diclosfenac sodium in rheumatoid arthritis. A correlation with serum interferon-like activity. Rev. Inter. Rheum. ("R") **79**, 215, 1985.

42. Mizrachi, Y., Rubinstein, M., Kimhi, Y. and Schwartz, M. Neurotrophic factor derived from goldfish brain: characterization and purification. J. Neurochem. **46**, 1675-1682, 1985.

43. Orchansky, P., Rubinstein, M., and Fischer, D.G. The interferon receptor in human monocytes is different from the one in non hematopoietic cells. J. Immunol. **126**, 169-173, 1986.

44. Goren, T., Fischer, D.G. and Rubinstein, M. Priming of leukocytes selectively increase the level of some interferon-α subtypes and not others. Biochim. Biophys. Acta **887**, 80-86, 1986.

45. Mory, Y., Ben-Barak, J., Segev, D., Cohen, B., Novick, D., Fischer, D.G., Rubinstein, M. and Revel, M. Efficient constitutive production of human IFN-γ. DNA **5**, 181-193, 1986.

46. Goren, T., Fischer, D.G. and Rubinstein, M. Human monocytes and lymphocytes produce a different mixture of interferon-α subtypes. J. Interferon Res. **6**, 323-329, 1986.

47. Fischer, D.G. and Rubinstein, M. Human monocyte tumoricidal activity. The role of IFN-γ and bacterial lipopolysaccharides in its stimulation, preservation and decay. Immunobiology **172**, 110-119, 1986.

48. Revel, M., Zilberstein, A., Ruggieri, M., Rubinstein, M. and Chen, L. Autocrine interferons and interferon β2. J. Interferon Res. **7**, 529-536, 1987.

49. Rubinstein, M. Multiple interferon subtypes: The phenomenon and its relevance. J. Interferon Res., **7**, 545-551, 1987.

50. Novick, D., Orchansky, P., Revel, M. and Rubinstein, M. The human interferon-γ receptor. Purification, characterization and preparation of antibodies. J. Biol. Chem. **262**, 8483-8487, 1987.

51. Fischer, D.G., Novick, D., Orchansky, P. and Rubinstein, M. Two molecular forms of the human interferon-γ receptor. J. Biol. Chem. **263**, 2632-2637, 1988.

52. Reiter, Z., Fischer, D.G. and Rubinstein, M. The protective effect of interferon against natural killing activity is not mediated via the expression of class I MHC antigens. Immunol. Lett. **17**, 323-328, 1988.

53. Chen, L., Novick, D., Rubinstein, M. and Revel, M. Recombinant interferon-β2 (interleukin-6) induces myeloid differentiation. FEBS Letters **239**, 299-304, 1988.

54. Shabo, Y., Lotem, J., Rubinstein, M., Revel, M., Clark S.C. Wolf, S.F., Kamen, R., Sachs, L. The myeloid blood cell differentiation inducing protein MGI-2 is interleukin-6. Blood. **72**, 2070, 1988.

55. Novick, D., Fischer, D.G., Reiter, Z., Eshhar, Z. and Rubinstein, M. Monoclonal antibodies to the human interferon-γ receptor: blocking of the biological activities of interferon-γ and purification of the receptor. J. Interferon Res. **9**, 315-328, 1989.

56. Engelmann, H., Aderka, D., Rubinstein, M., Rotman, D. and David Wallach. A tumor necrosis factor (TNF)-binding protein purified to homogeneity from human urine protects cells from TNF toxicity. J. Biol. Chem. **264**, 11974-11980, 1989.

57. Wallach, D., Holtmann, H., Aderka, D., Rubinstein, M., Shemer-Avni, Y., Sarov, I. and Engelmann, H. Mechanisms which take part in regulation of the response to Tumor Necrosis Factor. Lymphokine Res. **8**, 359-363, 1989.

58. Novick, D., Engelmann, H., Wallach, D. and Rubinstein, M. Soluble cytokine receptors are present in normal urine. J. Exp. Med. **170**, 1409-1414, 1989.

59. Edelbaum, O., Ilan, N., Grafi, G., Sher, N., Stram, Y., Novick, D., Sela, I. and Rubinstein, M. Two antiviral proteins from tobacco: Purification and characterization by monoclonal antibodies to human β-interferon. Proc. Natl. Acad. Sci. USA **87**, 588-592, 1990.

60. Reiter, Z. and Rubinstein, M. Interleukin-1α and Tumor Necrosis Factor-α protect cells against natural killer cell-mediated cytotoxicity and natural killer cytotoxic factor. Cellular Immunol. **125**, 326-336, 1990.

61. Lazard, D., Tal, N., Rubinstein, M., Khen, M., Lancet, D. and Zupko, K. Identification and biochemical analysis of novel olfactory-specific cytochrome P-450IIA and UDP-Glucuronosyl Transferase. Biochemistry **29**, 7433-7440, 1990.

62. Novick, D., Engelmann, H., Wallach, D., Leitner, O., Revel, M. and Rubinstein, M. Purification of soluble cytokine-receptors from normal human urine by ligand-affinity and immuno-affinity chromatography. J. Chromatography **510**, 331-337, 1990.

63. Yonish-Rouach, E., Shinitzky, M. and Rubinstein, M. A method for preparing biologically active aqueous cyclosporin A solutions avoiding the use of detergents or organic solvents. J. Immunol. Methods **135**, 147-153, 1990.

64. Harel, A., Fainaru, M., Rubinstein, M., Tal, N. and Schwartz, M. Fish Apolipoprotein A-I has heparin binding activity: Implication for nerve regeneration. J. Neurochemistry **66**, 1237-1243, 1990.

65. Novick, D., Engelmann, H., Revel, M. Leitner, O. and Rubinstein, M. Monoclonal antibodies to the soluble human IL-6 receptor: affinity purification, ELISA and inhibition of ligand binding. Hybridoma **10**, 137-146, 1991.

66. Yonish-Rouach, E., Fischer, D.G. and Rubinstein, M. Cyclosporin A regulates the expression of HLA-DR on human monocytes by two different mechanisms. Cellular Immunol. **134**, 402-413, 1991.

67. Yonish-Rouach, E., Kimchi, A. and Rubinstein, M. The antiproliferative effect of cyclosporin on hematopoietic and lymphoblastoid cell lines - common mechanistic elements with interferon alpha. Transplantation **51**, 1276-1282, 1991.

68. Reiter, Z., Reiter, Y., Fishelson, Z., Shinitzky, M., Kessler, A., Loyter, A., Nussbaum, O. and Rubinstein, M. Resistance to NK cell-mediated cytotoxicity (in K-562 cells) does not correlate with class I MHC antigen levels. Immunobiology, **183**, 23-29, 1991.

69. Lax, I., Mitra, A.K., Ravera, C., Hurwitz, D.R., Rubinstein, M., Ullrich, A., Stroud, R.M. and Schlessinger, J. Epidermal Growth Factor (EGF) induces oligomerization of soluble, extracellular, ligand-binding domain of EGF receptor. A low resolution projection structure of the ligand binding domain. J. Biol. Chem. **266**, 13828-13833, 1991.

70. Edelbaum, O., Sher, N., Rubinstein, M., Novick, D., Tal, N., Moyer, M., Ward, E., Ryals, J. and Sela, I. Two antiviral proteins, gp35 and gp22, correspond to β-1,3-glucanase and an isoform of PR-5. Pl. Mol. Biol. **17**, 171-173, 1991.

71. Merimsky, O., Rubinstein, M., Fischer, D., Danon, A. and Chaitchik, S. Pharmacokinetics of recombinant interferon alpha-C. Cancer Chem. Pharm. **27**, 406-408, 1991.

72. Mohammadi, M., Honegger, A.M., Rotin, D., Fischer, R., Bellot, F., Li, W. Dionne, C.A., Jaye, M., Rubinstein, M. and Schlessinger, J. A tyrosine-phosphorylated carboxy-terminal peptide of the fibroblast growth factor receptor (Flg) is a binding site for the SH2 domain of phospholipase C-γ1. Mol. Cell. Biol. **11**, 5068-5078, 1991.

73. Reiter, Z., Loyter, A., Nussbaum, O. and Rubinstein, M. An inducer of NKCF (NK cytotoxic factor) release: Localization on target-cell membrane and initial characterization. Cell. Immunol. **142**, 370-384, 1992.

74. Reiter, Z., Chen, L., Revel, M. and Rubinstein, M. Interleukin-6 protects ductal breast carcinoma cells from MHC-unrestricted cell-mediated cytotoxicity. Lymphokine and Cytokine Research **11**, 175-181, 1992.

75. Edelbaum, O., Stein, D., Holland, N., Gafni, Y., Livneh, O., Novick, D., Rubinstein, M. and Sela, I. Expression of active human interferon-β in transgenic plants. J. of Interferon Res. **12**, 449-453, 1992.

76. Novick, D., B. Cohen and M. Rubinstein. Soluble interferon-α receptor molecules are present in body fluids. FEBS Lett. **314**, 445-448, 1992.

77. Fischer, D.G., Tal, N., Novick, D., Barak, S. and Rubinstein, M. An antiviral soluble form of the LDL receptor induced by interferon. Science **262**, 250-253, 1993.

78. Zhou, M., Felder, S., Rubinstein, M., Hurwitz, D.R., Ullrich, A., Lax, I., and Schlessinger, J. Real-time measurements of EGF binding to soluble EGF receptor monomers and dimers support the dimerization model for receptor activation. Biochemistry **32**, 8193-8198, 1993.

79. Iacobelli, S., Bucci, I., D'Egidio, M., Giuliani, C., Natoli, C., Tinari, N., Rubinstein, M., and Schlessinger, J. Purification and characterization of a 90 kDa protein released from human tumors and tumor cell lines. FEBS Lett. **319**, 59-65, 1993.

80. Novick, D., Cohen, B., and Rubinstein, M. The Human Interferon α/β receptor: Characterization and molecular cloning. Cell **77**, 391-400, 1994.

81. Novick, D., Cohen, B., Tal N., and Rubinstein, M. Soluble and membrane-bound forms of the Human Interferon α/β receptor. J. Leukocyte Biol. **57**, 712-718, 1995.

82. Cohen, B., Novick, D., Barak, S., and Rubinstein M. Ligand-induced association of the Type I interferon receptor components. Molec. Cell. Biol. **15**, 4208-4214, 1995.

83. Domanski, P., Witte, M., Kellum, M., Rubinstein, M., Hackett, R., Pitha, P., Colamonici, O.R. Cloning and expression of a long form of the beta subunit of the interferon alpha beta receptor that is required for signaling. J. Biol. Chem. **270**, 21606-21611, 1995.

84. Cohen, B., Novick, D., and Rubinstein, M. Modulation of insulin activities by leptin. Science **274**, 1185-1188, 1996.

85. Gamus, D., Romano, A., Rubinstein, M., and Savion, N. Moderation of herpetic keratitis by basic fibroblast growth factor. Exp. Eye Res. **63**, 1-8, 1996.

86. Kim, S-H., Cohen, B., Novick, D., and Rubinstein, M. Mammalian Type I interferon receptors consist of two subunits: IFNaR1 and IFNaR2. Gene, 1997 **196**, 279-286, 1997.

87. Zelzer, E., Levy, Y., Kahana, C., Shilo, B-Z., Rubinstein, M., and Cohen, B. Insulin induces transcription of target genes through the hypoxia-inducible factor HIF-1 α/ARNT. EMBO J. **17**, 5085-5094, 1998.

88. Novick, D. Kim, S-H., Fantuzzi, G., Reznikov, L.L., Charles A. Dinarello, C.A., and Rubinstein, M. Interleukin-18 Binding Protein: A Novel modulator of the Th1 cytokine Response. Immunity **10**, 127-136, 1999.

89. Barkan, D., Jia, H., Dantes, A., Vardimon, L., Amsterdam, A., and Rubinstein, M., Leptin Modulates the Glucocorticoid-Induced Ovarian Steroidogenesis. Endocrinology **140**, 1731-1738, 1999.

90. Vidal-Vanaclocha F., Fantuzzi G., Mendoza L., Fuentes A.M., Anasagasti M.J., Martin J., Carrascal T., Walsh P., Reznikov L.L., Kim S-H., Novick D., Rubinstein M., Dinarello C.A. IL-

18 regulates IL-1beta-dependent hepatic melanoma metastasis via vascular cell adhesion molecule-1. Proc Natl Acad Sci U S A **97**, 734-739, 2000.

91. Kim S-H., Eisenstein M., Reznikov L., Fantuzzi G., Novick D., Rubinstein M., Dinarello C.A., Structural requirements of six naturally occurring isoforms of the IL-18 binding protein to inhibit IL-18. Proc Natl Acad Sci U S A **97**, 1190-1195, 2000.

92. Reznikov LL, Kim SH, Westcott JY, Frishman J, Fantuzzi G, Novick D, Rubinstein M, Dinarello CA. IL-18 binding protein increases spontaneous and IL-1-induced prostaglandin production via inhibition of IFN-gamma. Proc Natl Acad Sci U S A. **97**, 2174-2179, 2000.

93. Liu B-L., Kim S-H., Novick D. Rubinstein M. Production of a biologically active human interleukin 18 requires its prior synthesis as pro-IL-18. Cytokine. 2000 **12**, 1519-1525.

94. Novick, D. Nabioullin, RR., Ragsdale, W. McKenna, S., Weiser W., Garone, L., Burkins, C., Kim, S-H., Rubinstein, M., Tepper, MA., Arulanandam, ARN. The neutralization of Type I IFN biologic actions by anti-IFNAR2 monoclonal antibodies is not entirely due to inhibition of Jak-Stat Tyrosine phosphorylation. J. IFN & Cytokine Res. **20**, 971-982, 2000.

95. Kim S-H, Azam T, Yoon DY, Reznikov LL, Novick D, Rubinstein M, Dinarello CA. Site-specific mutations in the mature form of human IL-18 with enhanced biological activity and decreased neutralization by IL-18 binding protein. Proc Natl Acad Sci U S A. **98**, 3304-3309, 2001.

96. Cohen B., Barkan D., Levy Y., Goldberg I., Fridman E., Kopolovic J., Rubinstein M. Leptin Induces Angiopoietin-2 Expression in Adipose Tissues. J Biol Chem. **276**, 7697-7700, 2001.

97. Almog B., Gold R., Tajima K., Dantes A., Salim K., Rubinstein M., Barkan D., Homburg R. Lessing J.B., Nevo N., Gertler A., Amsterdam A. Leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats. Molec. Cell. Endocrin **183** 179-191. 2001.

98. Novick D., Schwartsburd B., Pinkus R., Suissa D., Belzer I., Sthoeger Z., Keane W.F., Chvatchko Y., Kim S-H., Fantuzzi G., Dinarello C.A., Rubinstein M., A Novel ELISA Shows elevated Serum IL-18BP in Sepsis and Extensive decrease of Free IL-18. Cytokine **14**, 334-342, 2001.

99. Arazi T., Slutsky S.G., Shiboleth Y.M., Wang Y., Rubinstein M., Barak S., Yang J., Gal-On A. Engineered Zucchini Yellow Mosaic Potvirus As A Non-Pathogenic Vector For Expression of heterologous Proteins in Cucurbits. J. Biotechnol. **87**, 67-82, 2001.

100. Zecchina G, Novick D, Rubinstein M, Barak V, Dinarello C, Nagler A. Interleukin-18 Binding Protein in Acute Graft versus Host Disease and Engraftment Following Allogeneic Peripheral Blood Stem Cell Transplants. J Hematother Stem Cell Res. **10**, 769-776, 2001.

101. Stuyt RJ, Netea MG, Kim SH, Novick D, Rubinstein M, Kullberg BJ, Van der Meer JW, Dinarello CA. Differential roles of interleukin-18 (IL-18) and IL12 for induction of gamma interferon by staphylococcal cell wall components and superantigens. Infect Immun. **69**, 5025-5030, 2001.

102. Kim SH, Azam T, Novick D, Yoon DY, Reznikov LL, Bufler P, Rubinstein M, Dinarello CA. Identification of amino acid residues critical for biological activity in human interleukin-18. J Biol Chem **277**, 10998-11003, 2002.

103. Ariel A, Novick D, Rubinstein M, Dinarello CA, Lider O, Hershkoviz R. IL-12 and IL-18 induce MAP kinase-dependent adhesion of T cells to extracellular matrix components. J Leukoc Biol. **72**, 192-198, 2002.

104. Kaser A, Novick D, Rubinstein M, Siegmund B, Enrich B, Koch RO, Vogel W, Kim SH, Dinarello CA, Tilg H. Interferon-alpha induces interleukin-18 binding protein in chronic hepatitis C patients. Clin Exp Immunol.;**129**, 332-338. 2002.

105. Yatsiv; I, Morganti-Kossmann; MC, Perez; D., Dinarello, CA, Novick, D., Rubinstein, M., Otto, VI., Rancan, M., Kossmann, T., Redaelli, CA., Trentz, O., Shohami, E., Stahel, PF. Elevated Intracranial IL-18 in Humans and Mice After Traumatic Brain Injury and Evidence of Neuroprotective Effects of IL-18-Binding Protein After Experimental Closed Head Injury. J. Cerebral Blood Flow & Metabolism. **22**, 971-978. 2002.

106. Shevah O, Nunez O, Rubinstein M, Laron Z. Intronic mutation in the growth hormone receptor gene in a Peruvian girl with Laron syndrome.J Pediatr Endocrinol Metab. **15**,1039-1040. 2002.

107. Sandowski Y, Raver N, Gussakovsky EE, Shochat S, Dym O, Livnah O, Rubinstein M, Krishna R, Gertler A. Subcloning, expression, purification, and characterization of recombinant human leptin-binding domain. J Biol Chem. **277**, 46304-46309. 2002.

108. Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Vogel W, Tilg H. Plasma levels of interleukin-18 and interleukin-18 binding protein are elevated in patients with chronic liver disease. J. Clin. Immunol. **22**, 331-337. 2002.

109. Hurgin, V., Novick, D., Rubinstein, M. The Promoter of IL-18 Binding Protein and its Activation by an IFNγInduced Complex of IFN Regulatory Factor-1 and CCAAT/Enhancer Binding protein β. Proc Natl Acad Sci U S A **99**, 16957-16962. 2002.

110. Stuyt, RJL., Kim, S-H,. Reznikov, LL., Fantuzzi, G., Novick, D., Rubinstein, M., Kullberg, BJ., van der Meer, JWM., Dinarello CA., Netea, MG. Regulation of Staphylococcus epidermidis-induced IFN-γ in whole human blood: the role of endogenous IL-18, IL-12, IL-1 and TNF. Cytokine, **21**, 65-73. 2003

111. Lonnemann G., Novick D., Rubinstein M., Passlick-Deetjen J., Lang D., Dinarello CA. A Switch to High-Flux Helixone((R)) Membranes Reverses Suppressed Interferon-gamma Production in Patients on Low-Flux Dialysis. Blood Purif. **21**, 225-231. 2003.

112. Dinarello CA, Novick D, Rubinstein M, Lonnemann G. Interleukin 18 and interleukin 18 binding protein: possible role in immunosuppression of chronic renal failure. Blood Purif. **21**, 258-270. 2003.

113. Gangemi S, Basile G, Merendino RA, Minciullo PL, Novick D, Rubinstein M, Dinarello CA, Balbo CL, Franceschi C, Basili S, Urbano ED, Davi; G, Nicita-Mauro V, Romano M. Increased circulating Interleukin-18 levels in centenarians with no signs of vascular disease: another paradox of longevity? Exp. Gerontol.. **38**, 669-672. 2003.

114. Shevah O, Borrelli P, Rubinstein M, Laron Z. Identification of two novel mutations in the human growth hormone receptor gene. J. Endocrinol. Ivest. **26**, 604-608, 2003.

115. Azam, T, Novick, D, Bufler, P, Yoon, D-Y, Rubinstein, M, Dinarello, CA, Kim, S-H, Identification of a Critical Ig-Like Domain in IL-18 Receptor and Characterization of a Functional IL-18 Receptor Complex. J. Immunol. **171,** 6574-6580, 2003.

116. Lonnemann G, Novick D, Rubinstein M, Dinarello CA. Interleukin-18, interleukin-18 binding protein and impaired production of interferon-gamma in chronic renal failure. Clin Nephrol. **60,** 327-334, 2003.

117. Feezor RJ, Oberholzer C, Baker HV, Novick D, Rubinstein M, Moldawer LL, Pribble J, Souza S, Dinarello CA, Ertel W, Oberholzer A. Molecular characterization of the acute inflammatory response to infections with gram-negative versus gram-positive bacteria. Infect Immun. **71,** 5803-5813, 2003.

118. Nolsoe RL, Pociot F, Novick D, Rubinstein M, Kim SH, Dinarello CA, Mandrup-Poulsen T. Mutation scan of a type 1 diabetes candidate gene: the human interleukin-18 binding protein gene. Ann N Y Acad Sci. **1005,** 332-339 2003.

119. Shevah O, Galli-Tsinopoulou A, Rubinstein M, Nousia-Arvanitakis S, Laron Z. Classical phenotype of Laron syndrome in a girl with a heterozygous mutation and heterozygous polymorphism of the growth hormone receptor gene. J Pediatr Endocrinol Metab. **17**, 371-374, 2004.

120. Shevah O, Rubinstein M, Laron Z. Molecular defects of the growth hormone receptor gene, including a new mutation, in Laron syndrome patients in Israel: relationship between defects and ethnic groups. Isr Med Assoc J. **6,** 630-633, 2004.

121. Kawashima M, Novick D, Rubinstein M, Miossec P. Regulation of interleukin-18 binding protein production by blood and synovial cells from patients with rheumatoid arthritis. Arthritis Rheum. **50,** 1800-1805, 2004.

122. Barkan D, Hurgin V, Dekel N, Amsterdam A, Rubinstein M. Leptin induces ovulation in GnRH-deficient mice. FASEB J. **19,** 133-135 2005.

123. Ludwiczek O, Kaser A, Novick D, Dinarello CA, Rubinstein M, Tilg H. Elevated systemic levels of free interleukin-18 (IL-18) in patients with Crohn's disease. Eur Cytokine Netw. **16**, 27-33 2005.

124. Shechter Y, Tsubery H, Mironchik M, Rubinstein M, Fridkin M. Reversible PEGylation of peptide YY3-36 prolongs its inhibition of food intake in mice. FEBS Lett. **579**, 2439-44 2005.

125. Neumayr G, Ludwiczek O, Helmut H, Pfister R, Mitterbauer G, Moschen A, Novick D, Rubinstein M., Tilg H. The Impact of Prolonged Strenuous Endurance Exercise on Interleukin 18 and Interleukin 18 Binding Protein in Recreational Cyclists. Int J Sports Med. **26**, 836-840 2005.

126. Mazodier K, Marin V, Novick D, Farnarier C, Robitail S, Schleinitz N, Veit V, Paul P, Rubinstein M, Dinarello CA, Harle JR, Kaplanski G. Severe imbalance of IL-18/IL-18BP in patients with secondary hemophagocytic syndrome. Blood. **106**, 3483-3489 2005.

127. Novick D, Rubinstein M, Azam T, Rabinkov A, Dinarello CA, Kim SH. Proteinase 3 is an IL-32 binding protein. Proc Natl Acad Sci U S A. **103**, 3316-3321, 2006.

CHAPTERS and PROCEEDINGS

1. Rubinstein, M., Wideman, J. and Stein, S. Isolation and analysis of opioid peptides from rat pituitary and guinea pig brain. In: Peptides, Proceedings of the Fifth American Peptide Symposium, M. Goodman and J. Meienhofer (Eds.) Eiley, N.Y. (1977) pp. 41-43.

2. Udenfriend, S., Rubinstein, M., and Stein, S. A reevaluation of the opioid peptides present in the central nervous system utilizing microfluorometric procedures. In: Endorphins in Mental Health Research (E. Usdin, Ed.) Macmillan Press, London, pp. 119-130, 1978.

3. Rubinstein, M., Rubinstein, S., Familletti, P.C., Hershberg, R.D., Brink, L.D., Gutter-man, J., Hester, J. and Pestka. S. Human leukocyte interferon. Production and purifica-tion to homogeneity by HPLC. Proc. 6th Amer. Peptide Sym. 99-103, 1979.

4. Rubinstein, M., Rubinstein, S., Familletti, P.C., Gutterman, J., Hester, J. and Pestka, S. An alternative source of human leukocyte interferon. In: Interferon, Properties and Clinical Uses. A. Khan, N.O. Hill, and G.L. Dorn (Eds.) Leland Fikes Foundation Press, Dallas (1979) pp. 45-58.

5. Evinger, M., Rubinstein, M. and Pestka, S. The growth inhibitory activity of human leukocyte interferon. In: Interferon, Properties and Clinical Uses. A. Khan, N.O. Hill and G.L. Dorn (Eds.) Leland Fikes Foundation Press, Dallas (1979) pp. 249-266.

6. Herberman, R.B., Ortaldo, J.R., Djeu, J.Y., Holden, T.H., Jett, J., Lang, N.P., Rubinstein, M. and Pestka, S. Role of interferon in regulation of cytotoxicity by natural killer cells and macrophages. Annals of the New York Academy of Sciences **350**, 63-71, 1980.

7. Evinger, M., Rubinstein, M. and Pestka, S. Growth-inhibitory and antiviral activity of purified leukocyte interferon. Annals of the New York Academy of Sciences **350**, 399-404, 1980.

8. Pestka, S., Evinger, M., McCandliss, R., Sloma, A. and Rubinstein, M. Human interferon: The messenger RNA and the proteins. In: Polypeptide Hormones. R.F. Beers, Jr. and E.G. Bassett (Eds.) Raven Press, N.Y. (1980) pp. 33-48.

9. Rubinstein, M. and Pestka, S. Purification and characterization of human leukocyte interferons by high performance liquid chromatography. In: Methods En-zymol. S.P. Colowick and N.O. Kaplan (Eds.) Academic Press, N.Y. Vol. **78** (1981) pp. 464-472.

10. Rubinstein, M. High performance liquid chromatography of interferon tryptic pep-tides at the subnanomole level. In: Methods Enzymol. S.P. Colowic and N.O. Kaplan (Eds.) Academic Press, N.Y. (1981) Vol. **79**, pp. 16-20.

11. Rubinstein, M., Stein, S. and Udenfriend, S. Fluorometric methods for analysis of proteins and peptides: principles and applications in: Hormonal proteins and peptides (Li C.H., ed.) Academic Press, pp. 1-24, 1980.

12 Moschera, J.A., Hobbs, D.S., Levy, W.P., Stein, S., Rubinstein, M. and Pestka, S. Purification and structure of the interferons. Texas Reports on Biology and Medicine **41**, 250-259, 1982.

13. Eshhar, Z., Novick, D., Gigi, Z., Marks, Z., Friedlander, Y., Revel, M. and Rubinstein, M. Monoclonal antibodies to human leukocyte and fibroblast interferon. In: Protides of Biological Fluid **30**, 491-494, 1983.

14. Orchansky, P., Goren, T. and Rubinstein, M. Isolated subtypes of human inter-feron-a: Synergistic action and affinity for the receptor. In: The Biology of the Interferon System, 1983. Elsevier Science Publishers B.V. (E. de Maeyer and H. Schellekens, eds) pp. 183-188.

15. Horovitz, O., Novick, D., Rubinstein, M. and Reel, M. Radio-immunoassays and Elisa for human leukocyte IFN-α. In: Immunoenzymatic Techniques, 1983, Elsevier Sciences Publishers, B.V. (S. Avrameas et al., eds.) pp. 193-196.

16. Novick, D., Eshhar, Z., Fischer, D., Mory, Y., Chernajovsky, Y., Revel, M. and Rubinstein, M. Monoclonal antibodies for purification and RIA of natural and recom-binant interferons-α, -β and -γ. In: Protides of the Biological Fluids (H. Peeters, ed.) Per-gamon Press, Oxford and New York, pp. 945-948, 1984.

17. Novick, D., Fischer, D.G., Mory, Y., Revel, M. and Rubinstein M. The use of ion ex-change and reversed phase HPLC for the isolation and characterization of IFN-γ. In: Protides of the Biological Fluids (H. Peeters. ed.) Pergamon Press, London and New York, pp. 1049-1052, 1984.

18. Mory, Y., Ben-Barak, J., Segev, D., Cohen, B., Novick, D., Fischer, D.G., Rubinstein, M. and Revel, M. Efficient constitutive production of human IFN-γ in mammalian cells. In: The Biology of the Interferon System (H. Kirchner and H. Schellekens, eds.) Elsevier Science Publishers, Amsterdam, 33-41, 1984.

19. Rubinstein, M., Fischer, D.G. and Orchansky, P. Multiple interferon gamma receptors. In: Interferons as Cell Growth Inhibitors and Antitumor Factors (R.M. Friedmann, T. Merigan and T. Sreevalsan, eds.), Alan R. Liss New York, pp 269-278, 1986.

20. Novick, D., Fischer, D.G., Reiter, Z., Eshhar, Z. and Rubinstein, M. Monoclonal antibodies to the interferon-γ receptor: Blocking of the biological activities of interferon-γ and purification of

the receptor. In: The Biology of the Interferon System (Y. Kawade and S. Kobayashi, eds.) Kodansha Scientific Ltd. Tokyo, 125-133, 1988.

21. Revel, M., Zilberstein, A., Chen, L., Gothelf, Y., Barash, I., Novick, D., Rubinstein, M., and Michalevicz, R. Biological activities of recombinant human IFN-β_2 /IL-6 (*E. coli*). In: Annals New York Acad. Sci. **557**, 144-156, 1989.

22. Novick, D., Engelmann, H., Wallach, D., Leitner, O., Revel, M. and Rubinstein, M. Soluble cytokine receptors in normal human urine: Purification, biological activity and monoclonal antibodies. In: Proceedings of 1st International Congress on Cytokines: Basic Principles and Clinical Applications. Raven Press. Vol. 68, 77-81, 1990.

23. Rubinstein, M., Revel, M., Novick, D., Mory, Y., Fischer, D., Kadouri, A., Betzalel, M., Zeevi, M., Hadas, E. and Erel, Z. Production, downstream processing and charac-terization of proteins from recombinant animal cells: the case of human interferon-β. In: Biologicals from recombinant microorganisms and animal cells production and recovery (M.D. White, S. Reuveni and A. Shafferman, eds.), VCH Weinheim, pp. 69-76, 1990.

24. Novick, D., Engelmann, H., Wallach, D. and Rubinstein, M. Soluble cytokine receptors are present in normal urine. In: Progress in Leucocyte Biology, Vol. 10B. The Physiological and Pathological Effects of Cytokines. Willey-Liss, Inc. New York, 413-421, 1990.

25. Engelmann, H., Aderka, D., Holtmann, H., Brakebusch, C., Nophar, Y., Novick, D., Kemper, O., Rubinstein, M., Shemer-Avni, Y., Sarov, I. and Wallach, D. Soluble forms of the TNF receptors, purified from human urine, inhibit TNF effects. Antibodies against the soluble receptors induce TNF-like effects. In: Molecular and Cellular Biology of Cytokines, Willey Inc. New York, 299-308, 1990.

26. Novick, D., Fischer, D.G. and Rubinstein, M. Purification of the human interferon-γ receptor by ligand affinity chromatography. In: Receptor Purification, The Human Press Inc., Clifton, New Jersey, pp. 459-481, 1990.

REVIEWS

1. Rubinstein, M., Stein, S. and Udenfriend, S. Fluorometric methods of analysis of proteins and peptides: Principles and applications (A review). In: Hormonal Proteins and Peptides, C.H. Li, (Ed.)., Academic Press, New York 9, 1-22, 1979.

2. Rubinstein, M. Purification and structural analysis of interferon. Phil. Trans. R. Soc. London B 299, 39-50, 1982.

3. Rubinstein, M. The structure of human interferons. Biochim. Biophys. Acta Reviews on Cancer 695, 5-16, 1982.

4. Orchansky, P. and Rubinstein, M. The Interferon Receptors. Critical Rev. Biochem. 21, 249-275, 1986.

5. Rubinstein, M., Novick, D. and Fischer, D.G. The Human IFN-γ Receptor System. Immunological Reviews. 97, 29-50, 1987.

6. Dinarello C.A, Novick D, Puren A.J, Fantuzzi G, Shapiro L, Muhl H, Yoon D-Y, Reznikov L.L, Kim S-H, Rubinstein M. 1998. Overview of interleukin-18: more than an interferon-gamma inducing factor. J. Leukoc. Biol. 63, 658-664, 1998.

PATENTS

1. Rubinstein, M. and Patchornik, A. Methods for the production of phosphate diesters. Israel patent application No. **47190**, April 28, 1975.

2. Rubinstein, M. Novel poly(ethylene oxide) derivatives. Israel patent application No. **47467**, June 12, 1975.

3. Rubinstein, M., Simon, S. and Block, R. A process for cross-linking of proteins. Israel patent application No. **47468**, June 12, 1975.

4. Perri, M., Rubinstein, M. and Kedem, O. Method and apparatus for the performance of selective electrodialysis. Israel patent application No. **49675**, May 28, 1976.

5. Pestka, S. and Rubinstein, M. Protein purification process and products. U.S. Patent No. **4,289,690**. September 15, 1981.

6. Friedlander, Y., Fischer, D.G. and Rubinstein, M. Purification of biologically active human interferon-gamma. Israel patent application No. 67896, February 13, 1983. U.S. patent No. **4,617,378**, October 14, 1986.

7. Friedlander, Y., Fischer, D.G. and Rubinstein, M. Interferon-gamma. Israel patent application No. 71688, April 24, 1984.

8. Revel, M., Mory, Y., Rubinstein, M. Production of interferon-gamma. Israel patent application No. 71691, April 27, 1984 (U.S. **727,672** and **727,673**, E.P.O. **85105083.3**, Australia, Canada, Japan and South Africa).

9. Revel, M. and Rubinstein, M. Constitutive production of human interferon-beta 1. U.S. patent application No. **669,259**, November 11, 1984.

10. Wallach D., Engelmann H., Aderka D., and Rubinstein, M. Tumor necrosis factor inhibitory protein and its purification. Israel paent application No 83878 Sep. 13, 1987. EP **0308378** Nov. 30th, 1994. US patent No **5,981,701**, Nov. 9, 1999.

11. Wallach D., Engelmann H., Aderka D., Novick, D., and Rubinstein, M. Tumor necrosis factor inhibitory protein and its purification. Israel paent application No 83878 Sep. 13, 1987. EP **0398327** March 15th, 1995.

12. Novick, D., Revel, M. Mory, Y., Rubinstein, M., and Hadas, E. IL-6-specific monoclonal antibodies, hybridomas therefor and methods of making and using same. Israel paent application No 83878 Nov. 14, 1988. US patent **5,618,700**, Apr 8th, 1997.

13. Novick, D., Mory, Y., Fischer, D. G., Revel, M. and Rubinstein, M. Interferon-gamma binding proteins. Israel paent application No 088378 Nov. 14, 1988, EP No. **0369413**, November 14, 1989. US patent **5,763,210**, Jun 9th, 1998.

14. Novick, D., Chen, L., Engelmann, H., Revel, M. Rubinstein, M. Wallach, D. Soluble IFN-β2/IL-6 binding protein its preparation, and pharmaceutical compositions containing it. US patent No. **5,621,077**, May 30, 1990.

15. Rubinstein, M., Novick, D. Tal N. Soluble LDL receptor, its production and use. Israeli patent application No. 100696, January 19, 1992. US patent No **5,723,438**, March 3th, 1998.

16. Rubinstein, M., Novick, D. Tal N. Soluble LDL receptor, its production and use. Israeli patent application No. 102915, Aug. 23, 1992. U.S. patent No. **5,496,926** March 5, 1996.

17. Novick, D. Rubinstein, M. An interferon-α/β binding protein, its preparation and pharmaceutical compositions containing it. Israeli patent application No. 103052, September 3,, 1992. European Patent No **0588177** 26 Jan. 2000.

18. Novick, D. Cohen, B. Rubinstein, M. Cloning of interferon-α/β binding protein. Israeli patent application No. 108584, February 7, 1994. U.S. patent No. **5,821,078** Oct. 13th, 1998. EP application 95101560.1

19. Barkan, D. Cohen, B. Novick, D., Rubinstein, M. Leptin as an inhibitor of tumor cell proliferation. Israeli patent application No. 120733, April 29, 1997.

20. Novick, D. Dinarello, C. Rubinstein, M. An IL-18 binding protein. Israeli patent application No. 121639, August 27, 1997.

CELL BIOLOGY

Signaling Vascular Morphogenesis and Maintenance

Douglas Hanahan

Blood vessels are constructed by two processes: vasculogenesis, whereby a primitive vascular network is established during embryogenesis from multipotential mesenchymal progenitors, and angiogenesis, in which preexisting vessels (both in embryo and adult) send out capillary sprouts to produce new vessels (*1*–*3*). Endothelial cells are centrally involved in each process: They migrate and proliferate and then assemble into tubes with tight cell-cell connections to contain the blood. Peri-endothelial support cells are recruited to encase the endothelial tubes, providing maintenance and modulatory functions to the vessels; such cells include pericytes for small capillaries, smooth muscle cells for larger vessels, and myocardial cells in the heart.

The establishment and remodeling of blood vessels is controlled by paracrine signals, many of which are protein ligands that bind and modulate the activity of transmembrane receptor tyrosine kinases (RTKs). This realization has emerged from the discovery and analysis of RTKs expressed on endothelial cells and of their ligands. Our basic view of RTK signaling has come from studies (performed largely in fibroblasts) of ligand-dependent autophosphorylation and activation of the branched Ras pathways. The results suggest that most RTKs are similarly coupled into the intracellular signal transduction cascade and are capable of inducing cell proliferation. However, the lessons from endothelial cells present a far more complicated picture. This complexity is highlighted by an article in this issue (*4*, page 55) of a ligand, called angiopoietin-2 (Ang2), that interferes with the kinase activity of an endothelial cell–selective RTK named Tie2. Remarkably, this inhibition of Tie2 kinase activity does not block endothelial cell proliferation and angiogenesis, but rather facilitates it.

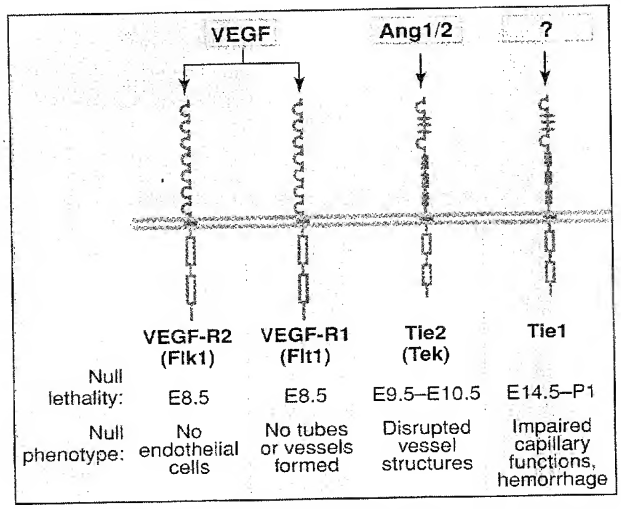

Fig. 1. Lessons from gene-knockout mice. The endothelial cell–selective RTKs VEGF-R1, VEGF-R2, Tie1, and Tie2 have all been ablated in gene-knockout mice. Each RTK knockout produced embryonic lethality with vascular defects. However, their distinctive phenotypes indicate that each of these tyrosine kinases controls a specific, complementary function in endothelial cells that collectively can account for a significant part of endothelial cell morphogenesis into functional vessels. See (*22*) for receptor structures.

Tie2 has two ligands, Ang1 and Ang2. Another major regulator of vasculogenesis and angiogenesis is vascular endothelial growth factor (VEGF, also called vascular permeability factor, VPF). VEGF signaling is itself mediated by two other endothelial cell–selective RTKs, called VEGF-R1 and VEGF-R2 (Flt1 and Flk1/KDR, respectively).

Mice carrying homozygous disruptions in the two highly homologous VEGF receptors die in mid-gestation of acute vascular defects, implicating each in vasculogenesis and developmental angiogenesis. However, the phenotypes are distinct—and instructive (see figure 1). VEGF-R2 knockout mice, which die by embryonic day 8.5 (E8.5), lack both endothelial cells and a developing hematopoietic system, implicating VEGF as a signal in the determination first of a hemato-angioblast progenitor and then of endothelial cells (*5*). In contrast, VEGF-R1 knockout mice, which also die around E8.5, have normal hematopoietic progenitors and abundant endothelial cells, which migrate and proliferate but do not assemble into tubes and functional vessels (*6*). Thus, these highly homologous RTKs send distinctive signals in endothelial cells.

Tie2 knockout mice die somewhat later in embryogenesis (E9.5 to E10.5). The Tie2 null phenotype is distinct from that of the VEGF receptor knockouts and is also informative. Endothelial cells are present in normal numbers and are assembled into tubes, but the vessels are immature, lacking branching networks and proper organization into large and small vessels (*7*, *8*). There is also an absence of the angiogenesis that vascularizes the neuroectoderm by capillary sprouting from the primitive vascular network (or plexus). Notably, the vessels that do form lack an intimate encapsulation by peri-endothelial support cells. In the heart the endocardium and myocardium do not show tight association and structura complexity; rather, the endocardial cell have aberrant, rounded shapes, are only loosely attached to the surrounding base ment membrane, and in many location are disconnected from myocardial cells Similar defects in vessel architecture are evident in other tissues. Thus, the Tie tyrosine kinase appears to control the ca pability of endothelial cells to recrui stromal cells to encase the endothelia tubes so as to stabilize the structure an modulate the function of blood vessels.

The fourth endothelial cell–selectiv RTK, Tie1, is remarkably similar in struc ture to Tie2 and appears to control an other aspect of vessel integrity. Knockou mice lacking Tie1 die over a variable pe riod, ranging from E14.5 to birth, of edem and hemorrhage, implicating the Tie1 signa in control of fluid exchange across capillari and in hemodynamic stress resistance (*8*, *9*

New insights into the surprising concep that the Tie2 RTK is primarily coupled int a signal transduction circuit that elicits ve sel maturation and maintains vessel integrit comes from functional analyses of th angiopoietins that bind to Tie2 and modu late its activity (*4*, *10*, *11*). The Tie2 ligan Ang1 and Ang2 are both ~75-kD secrete proteins with considerable sequence homo ogy; each contains a coiled-coil and a f brinogen-like domain. Both bind to the Ti receptor with similar affinity, and neith binds to the related receptor Tie1. Yet the effects on Tie2 are distinctive, as are the expression patterns in the mouse. Ang1 i duces autophosphorylation of Tie2 in cu tured endothelial cells. In marked contra Ang2, which binds with similar affinity, do not induce receptor phosphorylation. Rath it can competitively inhibit Ang1—induc kinase activation of the Tie2 receptor. Th

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Ang2 presents a negative signal to Tie2, a remarkable observation given its high homology to Ang1.

Moreover, this distinctive effect is apparently endothelial cell–specific. If a modified Tie2 is forcibly expressed in 3T3 fibroblasts, both Ang1 and Ang2 induce receptor phosphorylation and yet do not stimulate fibroblast proliferation. Similarly, Ang1-induced autophosphorylation of Tie2 does not affect endothelial cell growth in culture, consistent with the Tie2 knockout phenotype, which indicated that Tie2 is not required for endothelial cell proliferation during vasculogenesis. Functional studies in transgenic and gene-knockout mice support the notion that Ang1 signals Tie2 to recruit support cells, and that Ang2 inhibits this capability. Gene-knockout mice that lack Ang1 die with similar vascular defects to the Tie2 knockout mice (*11*). Transgenic mice overexpressing the negative ligand Ang2 in endothelial cells also die during embryogenesis, again with similar vascular defects (*4*). Thus, overexpression of Ang2 phenocopies loss of Ang1 expression, consistent with their opposite activities. Collectively, the data argue that Ang1 is the major physiological ligand for Tie2's functional role in recruiting and sustaining peri-endothelial support cells, whereas Ang2 serves to block this function, thereby relaxing these intimate and critical associations.

Why then does Ang2 exist? No doubt the Ang2 gene-knockout phenotype will prove illuminating. Meanwhile, the prevailing evidence suggests that Ang2 allows vascular remodeling, and in particular angiogenesis, processes that may be restricted by the encapsulation with basement membrane and peri-endothelial support cells. The clues come from the expression patterns of Ang1 and Ang2.

Ang1 is widely expressed both in the embryo and in the adult (*4, 10*). Ang2 is also widely expressed in the embryo. However, its expression pattern in the adult (in a limited survey) is provocative: Ang2 is selectively expressed in ovary, uterus, and placenta, the three tissues subject to physiological angiogenesis (*4*). The possible association of Ang2 with adult angiogenesis was investigated during ovulation, which is marked by distinctive phases of vascular quiescence, angiogenesis, and vascular regression. Ang2 expression in these stages was compared to that of Ang1 and VEGF. In early follicles, the vasculature is quiescent, and Ang1 is expressed, with little or no VEGF or Ang2 expression. In late pre-ovulatory follicles and in the postovulatory corpus luteum, where angiogenesis is ongoing, both VEGF and Ang2 are up-regulated, while Ang1 expression persists. Notably, Ang2 expression appears punctate, or focal. Finally, in nonproductive follicles, which show vascular regression, Ang2 is expressed at uniformly high levels, provocatively in the absence of VEGF expression.

These patterns of expression, when considered in the context of the lessons from the various gene-knockout phenotypes (see Fig. 1), collectively suggest a model for control of vasculogenesis, vessel maturation and maintenance, angiogenesis, and regression, as illustrated in Fig. 2. The Ang1/Tie2 circuit appears to mediate vessel maturation from simple endothelial tubes into more elaborate vascular structures composed of several cell types, and the maintenance of those vessels via the cell-cell and cell-matrix associations that produce them. Consequently, Ang1 may also help preserve endothelial cell quiescence. Focal expression of Ang2 evidently blocks the Ang1/Tie2 signal, resulting in a loosening of this tight vascular structure and thereby exposing the endothelial cells to activating signals from angiogenesis inducers, including VEGF. If VEGF (or another angiogenesis inducer) is present, the endothelial cells become activated to migrate and proliferate, producing new capillary sprouts and in turn tubes. One can envision that the more uniform presence of Ang1 allows a shift in the local balance of Ang1/Ang2 back in favor of Ang1, to effect maturation and stabilization of the newly formed vessels. Thus, there appears to be a collaboration between VEGF, Ang2, and Ang1 to elicit angiogenesis. In contrast, vascular regression is associated with very high-level expression of Ang2 in the absence of the activating (survival) signal from VEGF, presumably overwhelming the Ang1 signal and thereby producing catastrophic detachment from matrix and support cells, most likely with consequent apoptosis.

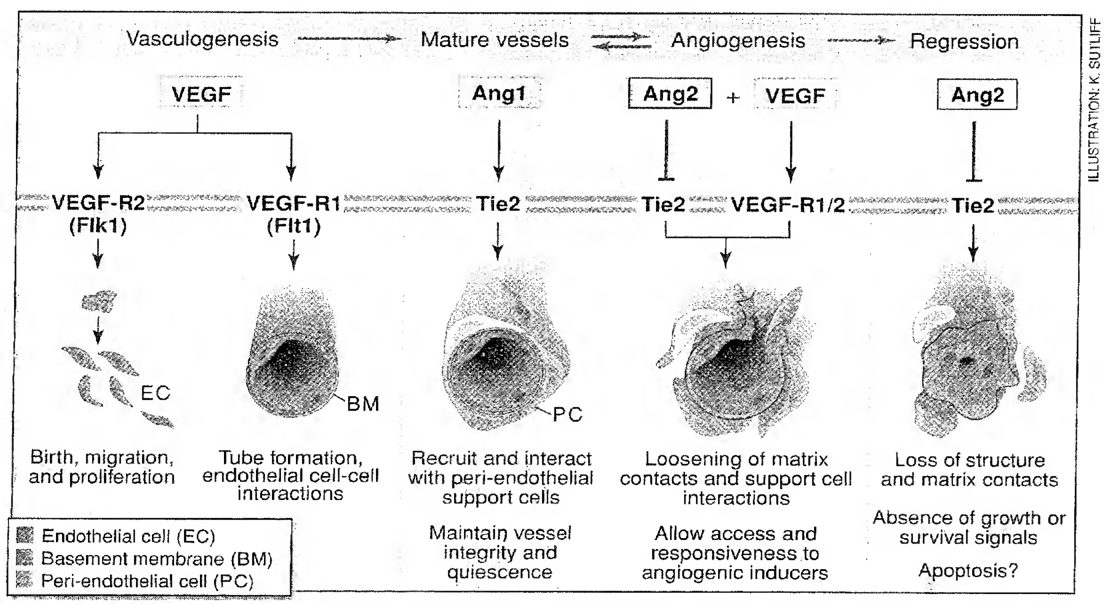

Fig. 2. Regulation of vascular morphogenesis, maintenance, and remodeling by RTKs and their ligands. A model for regulation of the vascular endothelium manifested by the prototypical angiogenesis factor VEGF and a new class of angiogenic regulators, Ang1 and Ang2. All three ligands bind to RTKs that have similar cytoplasmic signaling domains. Yet their downstream signals elicit distinctive cellular responses. Only VEGF binding to the VEGF-R2 sends a classical proliferative signal. When first activated in embryogenesis, this interaction induces the birth and proliferation of endothelial cells. In contrast, VEGF binding to VEGF-R1 elicits endothelial cell-cell interactions and capillary tube formation, a process that follows closely proliferation and migration of endothelial cells. Ang1 binding to the Tie2 RTK recruits and likely maintains association of peri-endothelial support cells (pericytes, smooth muscle cells, myocardiocytes), thus solidifying and stabilizing a newly formed blood vessel. The newly discovered Ang2, although highly homologous to Ang1, does not activate the Tie2 RTK; rather, it binds and blocks kinase activation in endothelial cells. The Ang2 negative signal causes vessel structures to become loosened, reducing endothelial cell contacts with matrix and disassociating peri-endothelial support cells. This loosening appears to render the endothelial cells more accessible and responsive toward the angiogenic inducer VEGF (and likely to other inducers). Finally, Ang2 is expressed at uniformly high levels in vascular regression in nonproductive ovarian follicles; the lack of VEGF coexpression suggests that loosening of cell-matrix interactions in the absence of a growth or survival signal elicits endothelial cell death, likely by apoptosis. No doubt additional factors play into these distinctive states, including the emerging class of angiogenesis inhibitors that directly block endothelial cell proliferation and migration.

The work by Maisonpierre *et al.* (*4*) introduces an inhibitory ligand, Ang2, for the Tie2 regulatory RTK that normally helps maintain vascular integrity. A model is emerging of a regulatory mechanism through which blood vessels are constructed, maintained, remodeled, and eliminated. Other factors are also involved, during embryonic vasculogenesis and angiogenesis, and for physiological and pathological angiogenesis in the adult. For example, gene-knockout mice have also implicated a G protein–coupled receptor in vascular regulation, because in the absence of $G_\alpha 13$, embryos die at E8.5 to E9.5, with defects in vascular assembly and angiogenesis (*12*). Moreover, a reciprocal paracrine signal has been revealed by gene-knockout mice that lack the genes for neuregulin, which is expressed in the endocardium of the heart, and the ErbB-2/3/4 RTKs, which are expressed in myocardium. Knockouts of this ligand or of its receptors produces defects in the developing heart analogous to those observed when Ang1 or Tie2 is missing (*13–16*). A similar role is suggested by gene-knockout mice for platelet-derived growth factor and its receptors in other tissue vasculature (*17*, *18*). Furthermore, there is clear evidence in the adult for additional angiogenesis inducers and for an increasing number of angiogenesis inhibitors that act directly on endothelial cells (*19–21*). Thus, regulation of angiogenesis in ovulation and implantation, in wound healing, and in chronic pathological situations such as tumor progression will indeed be complex, but tractable using the power of animal models.

That complexity notwithstanding, the evidence is compelling that VEGF and the angiopoietins, and their cognate receptors, are critical components of the vascular regulatory machinery. It will be of particular interest to establish the possible contributions of Ang2 to tumor angiogenesis, whereby the quiescent vasculature (likely maintained in part by Ang1/Tie2) is activated to elicit and chronically effect the angiogenic phenotype that accompanies tumor growth and metastasis. Ang2 could well serve as an initial angiogenic signal, locally opening up the vessel structure to allow protease degradation of the basement membrane surrounding the endothelium and accessibility to that endothelium by angiogenesis inducers such as VEGF, thereby eliciting capillary sprouting and in turn new blood vessels that sustain a tumor as it expands.

References and Notes

1. W. Risau and I. Flamme, *Annu. Rev. Cell Dev. Biol.* **11**, 73 (1995).
2. J. Folkman and P. A. D'Amore, *Cell* **87**, 1153 (1996).
3. W. Risau, *Nature* **386**, 671 (1997).
4. P. C. Maisonpierre *et al.*, *Science* **277**, 55 (1997).
5. F. Shalaby *et al.*, *Nature* **376**, 62 (1995).
6. G.-H. Fong, J. Rossant, M. Gertsenstein, M. L. Breitman, *ibid.*, p. 66.
7. D. J. Dumont *et al.*, *Genes Dev.* **8**, 1897 (1994).
8. T. Sato *et al.*, *Nature* **376**, 70 (1995).
9. M. C. Puri, J. Rossant, K. Alitalo, A. Bernstein, J. Partanen, *EMBO J.* **14**, 5884 (1995).
10. S. Davis *et al.*, *Cell* **87**, 1161 (1996).
11. C. Suri *et al.*, *ibid.*, p. 1171.
12. S. Offermanns, V. Mancino, J.-P. Revel, M. I. Simon, *Science* **275**, 533 (1997).
13. K.-F. Lee *et al.*, *Nature* **378**, 394 (1995).
14. M. Gassmann *et al.*, *ibid.*, p. 390.
15. D. Meyer and C. Birchmeier, *ibid.*, p. 386.
16. R. Kramer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 4833 (1996).
17. P. Leveen *et al.*, *Genes Dev.* **8**, 1875 (1994).
18. P. Soriano, *ibid.*, p. 1888.
19. N. Bouck, V. Stellmach, S. Hsu, *Adv. Cancer Res.* **69**, 135 (1996).
20. J. Folkman, in *The Molecular Basis of Cancer*, J. Mendelsohn, P. M. Howley, M. A. Israel, L. A. Liotta, Eds. (Saunders, Philadelphia, 1995), vol. 9, pp. 206–232.
21. D. Hanahan and J. Folkman, *Cell* **86**, 353 (1996).
22. T. Mustonen and K. Alitalo, *J. Cell Biol.* **129**, 895 (1995).
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CONDENSED MATTER PHYSICS

Low-Energy Excitations in High-Temperature Superconductors

Patrick A. Lee

Many common metals undergo a phase transition at some low critical temperature T_c and become superconductors. This phenomenon was explained in the famed BCS (Bardeen-Cooper-Schrieffer) theory (*1*) in terms of the coupling of electrons into Cooper pairs. In these materials, the superconducting ground state gains binding energy as a result of the pairing. It costs a finite amount of energy, called the energy gap, to break up the pairs to form excitations called quasiparticles. These are quantum mechanical superpositions of the electron and the hole excitations of the metallic state. These quasiparticles are interesting objects in their own right, but at low temperatures their number is exponentially small, and they hardly make their presence felt.

Until a decade ago, it was generally believed that the relative angular momentum of the electrons in Cooper pairs is *s* wave. Consequently, the energy gap is independent of the momentum **k** of the quasiparticle. This independence is in contrast to the only other case of Cooper pairing between fermions known at the time, namely, the superfluid state in liquid ^{3}He (*2*). There the pairing state is anisotropic (*p* wave), and the energy gap may vanish along some momentum direction. These nodal points in the energy gap play an important role because quasiparticles near the nodes can be created in large numbers and they are responsible for the low-temperature properties. Today the prevailing view is that in high-T_c superconductors, the Cooper pairs are formed in the *d*-wave state. The role of the quasiparticles i then more analogous to that in superfluic ^{3}He, and experimentalists are beginning to study them in detail. On page 83 of this issue Krishana *et al.* (*3*) report a particularly interesting example of this class of experiments.

Although the *s*-wave BCS pairing theor works perfectly for practically all metallic superconductors, even a decade ago there was suspicion that a few superconductors are exceptions. The main evidence was that the low-temperature properties are not expo nentially activated, pointing to the exist ence of nodes in the energy gap. The sus pected unconventional superconductor were the so called "heavy fermion" supercon ductors (*4*) containing rare-earth ions sucl as Ce or U and a number of organic supercon ductors (*5*). The field of unconventional su perconductivity took a dramatic turn in 198 with the discovery of high-temperature su perconductivity in cuprates (*6*). Apart fror the high T_c, the metallic state (and to a lesse extent, the superconducting state) of thes materials is so unusual that it was widel speculated that the pairing state is not th conventional *s* wave. After a number of bri liant experiments (*7*), it is now widely ac cepted that the Cooper pairs in the cuprate are formed in the *d*-wave symmetry. Th symmetry implies that the energy gap var ishes at four points on the Fermi surface c these two-dimensional materials. Fro

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